TITLE OF THE INVENTION:

Biochip Measuring Method and Measuring Equipment

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a biochip reader for measuring a plurality of types of biopolymers on a substrate, and in particular, relates to an improvement for enabling the measurement of a wider area on a substrate while maintaining a large numerical aperture.

2. Description of the Prior Art

There is well-known equipment which detects and analyze DNA or protein by labeling biopolymers such as DNA or protein with fluorescent materials, exciting those fluorescent materials through irradiation of the biopolymers with laser, and reading the fluorescence generated from the fluorescent materials. In this case, biochips on which DNA or protein or the like labeled with fluorescent materials is spotted in an array are utilized.

Fig. 1 is a conceptual configuration drawing showing an example of conventional incident-light fluorescence biochip readers mentioned in the gazette of Japanese Laid-open Patent Application No. 2000-207007. This biochip reader reads hybridization of unknown gene α as shown in Fig. 1 (b) and biochip 6 composed of a plurality of DNA molecules (genes) A, B, C, ... whose sequences are known bonded on substrate PL as shown in Fig. 1 (a) using a mechanism as shown in Fig. 1 (c).

In Fig. 1 (c), light from light source 1 (laser) becomes the parallel light at lens 2 and, after transmitting dichroic mirror 4, is focused on biochip (or called a sample) 6 by means of lens 3. The light returned from biochip 6 becomes parallel again by means of lens 3 and is reflected with dichroic mirror 4 and forms an image on camera 9 by means of lens 8.

In this case, the surface of biochip 6 is scanned by moving the stage (not shown in the drawing) on which biochip 6 is mounted in the directions of X and Y using a driving means (not shown in the drawing) to obtain the image of the surface of biochip 6.

However, there are the following problems with such conventional systems:

(1) Fig. 2 is a drawing for the optical system shown in Fig. 1. The measurable range is determined by the CCD camera used and magnifications of lenses 3 and 8 and the following relations exist between them:

$$a_1/a_2 = f_1/f_2 = NA_2/NA_1$$

where a_1 is the width of measurement area (field of view of camera 9) of biochip 6.

a_2 is the width of the detecting element surface of camera 9.

f_1 is the focal length of lens 3.

f_2 is the focal length of lens 8.

NA_1 is the numerical aperture of lens 3.

NA_2 is the numerical aperture of lens 8.

Due to these relations, if measurement area a_1 is widened, the image becomes dark because the incident NA_1 becomes small.

(2) When the size of the detecting element, CCD, of camera 9 is, for example, 1/2 inch, its field of view is about 4.8×6.4 mm^2. This value is about 1/60 smaller than the measurement area of 75×25 mm^2 in the case where sample 6 is, for example, a slide glass. Furthermore, in the case of the system where conventional one-beam laser irradiates a sample which is moved with a stage, and the total light quantity for each step is detected with photomultipliers or the like, a precision stage is required and so the system is expensive and measurement is time-consuming. If it is assumed that measurement is made in about 10-μm step using one-beam exciting light, a measurement area of 75×25 mm^2 must be measured by moving the stage 1.875×10^7 times.

SUMMARY OF THE INVENTION

The present invention is intended to solve the above-described problems; the objective is to achieve a measuring method and measuring equipment for biochips that can measure images in bright conditions over a wide biochip measurement area.

BRIEF DESCRIPTION OF THE DRAWINGS

[Fig. 1]

Fig. 1 is a conceptual configuration drawing showing an example of conventional incident-light fluorescence biochip readers.

[Fig. 2]

Fig. 2 is a drawing illustrating the relationships in the optical system of the above example.

[Fig. 3]

Fig. 3 is a principle diagram describing the principle of the biochip measuring method of the present invention.

[Fig. 4]

Fig. 4 is a drawing illustrating jumped movement.

[Fig. 5]

Fig. 5 is a drawing indicating the configuration of the essential part in the case of using a line camera of one-dimensional array.

[Fig. 6]

Fig. 6 is a drawing showing another embodiment of step movement.

[Fig. 7]

Fig. 7 is a drawing illustrating the position where images of the field-of-view images are joined.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The biochip measuring method or measuring equipment of the present invention is a measuring method or measuring equipment which measures a plurality of types of biopolymers on a substrate using fluorescence or colorimetric means. The present invention will be described below in detail using the drawings.

Fig. 3 is a principle diagram describing the principle of the biochip measuring method of the present invention. In Fig. 3, the items equivalent to those shown in Fig. 1 are given the same signs. If measurement area (X_2, Y_2) 10 of sample 6 is wider than the field of view (X_1, Y_1) of camera 9, a plurality of images is photographed with camera 9 over the whole measurement area by moving sample 6 step-wise by an integer multiple of the above field of view using a stage (not shown in the drawing). Then, the entire image is made by combining that plurality of images (called the field-of-view

images) using an image processing means (not shown in the drawing).

For example, if X_2 and Y_2 of measurement area (X_2, Y_2) 10 are 75 mm and 25 mm respectively, the relationships between the CCD size (and the size of the field of view in that case) and the number of times of step-wise movement are as shown in the table below.

Table 1

CCD	Field of View	Number of Times of Movement
1/2 inch	X1 = 6.4 mm Y1 = 4.8 mm	X direction: 12 times Y direction: 6 times
1/3 inch	X1 = 4.8 mm Y1 = 3.6 mm	X direction: 16 times Y direction: 7 times
1/5 inch	X1 = 2.95 mm Y1 = 2.21 mm	X direction: 26 times Y direction: 12 times

In this case, border parts of images adjacent to each other are first measured in an overlapped manner and then, if there are shifts, those positions are corrected within the image plane. In addition, if there is unevenness of light quantity in the image plane, the unevenness is corrected. These corrections are implemented in the image processing means.

Furthermore, the present invention is not restricted to the above embodiment but may be embodied in other specific forms, changes, and versions without departing from the spirit or essential characteristics thereof.

For instance, for the above described stage moving mechanism, mechanisms which are moved using an electromagnetic drive, electrostatic drive, piezo-electric drive, or the like, can be used.

In image measurement, step movement involving jumping over non-sample areas 11 in measurement area 10 (that is, jumping movement) as shown in Fig. 4 may be employed.

Further, as shown in Fig. 5, measurement may be made by moving sample 6 step-wise in the direction orthogonal to the camera array direction by means of single-shaft driving, using line camera 31 in which detector elements are arrayed one-dimensionally as a camera to be employed.

Movement relative to the field of view on sample 6 is not restricted

to the movement in the orthogonal direction as in the embodiment described above but may be rotational movement such as ringed or helical as shown in Fig. 6. In this case, each measurement area 61 should also be arranged ringed or helically.

Further, for step movement, the prescribed measurement area can be covered within 50 steps on each axis of Cartesian coordinates or polar coordinates.

In addition, when images are to be combined, the combination is not made so that adjacent images are overlapped on an image of site 63 in field-of-view image 62 as shown in Fig. 7 (a) but it is made at the end (boundary) of field-of-view image 62. In other words, the combination of images can be facilitated by ingeniously utilizing the fact that there are gaps between sites (DNA spots).

As described above, the present invention has the following effects:

(1) Biochips must be measured at high sensitivity because of trace amounts of expressed genes or the like. This requires a large numerical aperture (NA). According to the present invention, images can be easily measured over a wide area while maintaining the large NA.

(2) In measuring the entire measurement area, a far smaller number of movements is required than in conventional stage scans. Accordingly, it is sufficient for the purpose of measurement to employ simpler, cheaper moving mechanisms.